REMARKS

The status of the application is as follows:

Original Claims 1-12 were presented for prosecution.

Original Claims 7 and 12 were withdrawn from consideration by the Examiner as being drawn to non-elected subject matter, and were cancelled by the Applicant.

Original Claims 1-6 and 8-11, as previously amended, were rejected by the Examiner.

Original Claims 1-6 and 8-11 were previously amended and are further amended or herein.

Previously canceled Claim 12 has been reinstated and amended herein.

No Claims have been allowed.

Claims 1-6 and 8-12, as amended, are pending for reconsideration by the Examiner.

In a Notice of Non-compliant Amendment, mailed February 26, 2008, and referring to MPEP §714 [R-6] II and 37 CFR §1.121. Part C, the Examiner noted that "[a] claim being canceled must be indicated as 'canceled;' and the text of the claim must **not** be presented."

In an Interview Summary, mailed February 26, 2008, the Examiner advised that:

"An allowance conference was held 2/14/2008. Applicant was informed of the following issues resulting from the allowance conference as a display of good customer service:

(1) The amendment filed 11/06/2007 is non-compliant due to the presence of text in canceled claims.

(2) The priority is not perfected in the specification.

(3) Possible new grounds of rejections (enablement) were discussed regarding species of effector signals (claims 4 and 5), and regarding cooperative and reversible language (claim 11).

(4) The Terminal Disclaimer filed 11/6/2007 was not in proper form, as it was filed for application 11/670,966.

(5) Applicant was reminded that claim 12 (currently [sic] cancelled) is drawn to a method for making the product of claim 1, and therefore could not be reinstated. However, a new claim drawn to a method of making the product of claim 1 would be rejoined at the time of allowance if the product of claim 1 was found to be allowable."

In response to item (1) of the Interview Summary, Applicants have herein removed the objectionable text following the identification, in parentheses, of Claims 6, 7, 8, 9 as "canceled".

In response to item (2) of the Interview Summary, Applicants respectfully refer the Examiner to the Preliminary Amendment, filed on July 13, 2004, which

contained the following instruction:

> "Please amend the specification as follows:
>
> Page 1, between "Title" and "Background of the Invention" headings insert:
>
> **Related Application**
>
> The present invention claims priority to U.S. Provisional No. 60/456,965 filed on March 21, 2003, which is incorporated herein by reference in its entirety.
>
> **Statement Regarding Federally Sponsored Research & Development**
>
> Some of the research described in this application was funded by a grant (5ROIGM5700904) from the National Institutes of Health and a grant from the Department of Defense (DAMDI79818558). The U.S. government may therefore have certain rights in the invention."

In response to item (3) of the Interview Summary:

- Applicants have herein amended Claims 4 and 5 to delete "chemical denaturants and "mutations" from the group of "controllable effector signals" claimed in Claim 4 and Claim 5; and,

- Applicants have herein amended Claim 11 to delete the term "cooperative and reversible conformational equilibrium" replacing it with the term "either a folded or unfolded conformation", consistently with the language used in Claim 2, as previously amended.

In response to item (4) of the Interview Summary, Applicants respectively submit herewith a corrected Terminal Disclaimer in proper form to replace the Terminal Disclaimer filed on 11/6/2007.

In response to item (5) of the Interview Summary, Applicants have reinstated previously withdrawn and canceled claim 12, and have amended it to refer a method of producing the fusion protein of claim 1

CONCLUSION

Applicants respectfully believe that all independent claims and dependent claims now meet the acceptance criteria for allowance and therefore request favorable action. If the Examiner believes that anything further would be helpful to place the application in better condition for allowance, Applicants invite the Examiner to contact Applicants' representative at the telephone number listed below.

Respectfully submitted,

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APPENDIX A:

"Clean" Version of Claim Listing

1. (Previously Amended) A fusion protein comprising a ubiquitin insert protein having an insert regulatory domain lying between an amino terminal and a carboxyl terminal of the ubiquitin insert protein; and, a barnase target protein having a surface loop that begins at an alpha carbon of an initial amino acid of the surface loop and terminates at an alpha carbon of a terminal amino acid of the surface loop, the surface loop comprising a cytotoxic target domain of the barnase target protein, wherein, the ubiquitin insert protein is inserted at a point within the surface loop between the alpha carbon of the initial amino acid of the surface loop and the alpha carbon of the terminal amino acid of the surface loop, such that an amino-carboxyl length of the ubiquitin insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the barnase target protein.

2 (Previously Amended) The fusion protein of claim 1, wherein the insert regulatory domain exists in either a folded or unfolded conformation and the target cytotoxic domain exists in either a folded or unfolded conformation, the insert regulatory domain and the target cytotoxic domain comprising a cooperative and reversible conformational equilibrium such that if the insert regulatory domain is in its folded conformation, the target cytotoxic domain is in its unfolded conformation and vice versa.

3. (Previously Amended) The fusion protein of claim 2, wherein the target cytotoxic domain folds under the influence of a first controllable effector signal and the insert regulatory domain folds under the influence of a second controllable effector signal.

4. (Currently Amended) The fusion protein of claim 3, wherein the first controllable effector signal is selected from the group comprising ligand binding, pH, and temperature in either the insert domain or the target domain.

5. (Currently Amended) The fusion protein of claim 3, wherein the second controllable effector signal is selected from the group comprising ligand binding, pH, and temperature in either the insert domain or the target domain.

6. (Currently Canceled)

7. (Previously Canceled)

8. (Currently Canceled)

9. (Currently Canceled)

10. (Previously Amended) The fusion protein of claim 2, wherein the insert protein comprises human ubiquitin, the insert regulatory domain comprises a regulatory domain of human ubiquitin, the target protein comprises barnase, the target cytotoxic domain comprises a cytotoxic domain of barnase, the amino-

carboxyl length is about 38 Å, the initial amino acid of the surface loop comprises proline in the number 64 position ("Pro64"), the terminal amino acid of the surface loop comprises threonine in the number 70 position ("Thr70"), and the alpha-carbon-alpha-carbon length is about 10.4 Å.

11. (Currently Amended: CLEAN) The fusion protein of claim 10 wherein the regulatory domain of human ubiquitin and the cytotoxic domain of barnase comprise either a folded or unfolded conformation which folded or unfolded conformation is subject to the influence of the controllable first and second effector signals.

12. (Currently Amended) A method for the production of the fusion protein of claim 1 comprising the steps of:

a. selecting a linker containing first and second restriction sites between a Lys66 and a Ser67 codon of a barnase gene;

b. using said first and second restriction sites of said linker to operationally insert a ubiquitin gene between two amino-acid codons of said linker, thereby creating a ubiquitin-barnase fusion gene;

c. fully sequencing said ubiquitin-barnase fusion gene to verify its integrity;

d. using enzymes to operationally insert said ubiquitin-barnase fusion gene into any plasmid of a BL21 (DE3) family, thereby creating an interim ubiquitin-barnase fusion expression plasmid;

e. operationally inserting a gene for barstar and its natural promoter from *Bacillus amyloliquifaciens* into said interim ubiquitin-barnase fusion expression plasmid,

thereby creating a ubiquitin-barnase fusion-barstar complex plasmid;

f. cloning said gene for barstar into a T7 promoter-containing plasmid conferring resistance to an antibiotic other than ampicillin onto cells transformed by said T7 promoter-containing plasmid, thereby creating a barstar plasmid;

g. transforming *E. coli BL21* (DE3) cells grown at about 20 to 37 degrees C in any medium compatible with E. coli growth using both said barstar plasmid and said ubiquitin-barnase fusion-barstar complex plasmid, and inducing said *E. coli* BL21 (DE3) cells with about 100 mg/L isopropyl b-D-thiogalactopyranoside;

h. harvesting said transformed *E. coli* cells by centrifugation after about 2 to 12 hours; after said induction;

i. placing said harvested *E. coli* cells in10 mM sodium phosphate at a pH of 7.5, thereby creating a solution of harvested *E. coli* cells;

j. lysing said solution of harvested *E. coli* cells by repeated freeze-thaw cycles in the presence of about 10mg/liter lysozyme, thereby creating a lysate;

k. adding about 10 mg/liter DNase I to reduce the viscosity of said lysate;

l. centrifuging said reduced viscosity lysate to remove insolubles, thereby forming a supernatant;

m. adding about 8 M urea to said supernatant to dissociate bound barstar;

n. Removing said dissociated barstar from said supernatant by passing said supernatant through an anion exchange chromatography resin to yield a solution;

o. loading said solution onto a cation exchange column;

p. washing said solution with about 10 mM sodium phosphate (pH about 7.5)

and about 6 M urea;

q. eluting said solution using a 0 to 0.2 M NaCl gradient;

r. Removing said urea from said dilution by dialysis against double-distilled water to

APPENDIX B

"Clean" Version of Amendment to Paragraph [0034] of the Specification

[0034] The tertiary structure of a protein may contain a surface loop. A surface loop is a continuous length of polypeptide chain whose constituent amino acids are in neither an alpha helical conformation nor in a beta sheet conformation, and can contact at least five water molecules, as determined by the DSSP computer program of Wolfgang Kabsch and Chris Sander. The DSSP, a program which is well known in the art, defines secondary structure, geometrical features and solvent exposure of proteins, given atomic coordinates in Protein Data Bank format, which is also well known in the art. (W. Kabsch & C. Sander, "Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical figures", Biopolymers 22, 2577-2637.